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Chemotherapeutic Agent-induced Nephrotoxicity in Rabbits: Protective Role of Grape Seed Extract

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Abstract: Cisplatin (CP) is one of the most effective cancer chemotherapeutic agent used against various solid tumors. This study was designed to investigate the antioxidant effects of Grape Seed Extract (GSE) on CP induced oxidative damage in rabbit kidney. The material of study was consist of 18 male New Zealand rabbit. Animals were divided into 3 groups. The first group was administered a single intraperitoneal (i.p.) dose of 0.9% saline. The second group of rabbits was treated with CP (a single i.p. dose of 5 mg kg⁻¹ body weight); The third group of rabbits was treated with GSE by gavage at the dose of 250 mg kg⁻¹ body weight for 6 consecutive days before and 6 consecutive days after a single i.p. CP injection. As a result of CP administration, kidney function tests; urea, BUN (Blood Urea Nitrogen) and creatinine levels were increased (p<0.001). It was observed that CP increase malondialdehyde (MDA) levels, the most important indicator of oxidative damage and decreased the activity of catalase (CAT). Administration of CP did not caused statically a change in glutathione (GSH) levels and glutathione peroxidase (GSH-P_x) activity (p>0.05). GSE treatment has high levels of MDA to compared to normal levels (p<0.001) but not an increase in CAT (p<0.05). In CP treatment group, severe acute tubular necrosis was seen. It was determined that in CP+GSE group mild degenerative changes were received instead of acute tubular necrosis. GSE has been found to be partially protective effect against CP induced biochemical and histopathological changes in rabbit kidney.

Key words: Cisplatin, grape seed extract, nephrotoxicity, rabbit

INTRODUCTION

Cisplatin is a highly effective antineoplastic drug commonly used for treatment of wide variety of solid tumors (Tayem *et al.*, 2006; Abdin *et al.*, 2008). However, the clinical usefulness of this drug is limited by the development of nephrotoxicity, a side effect that may be produced in various animal species (Kim *et al.*, 1995). Primary targets of cisplatin in kidneys are proximal straight and distal convoluted tubules, where it accumulates and promotes cellular damage and by involving multiple mechanisms including oxidative stress, DNA damage, apoptosis and inflammation (Mohan *et al.*, 2006; Schaff *et al.*, 2002). The exact mechanisms of nephrotoxicity induced by cisplatin are still not fully elucidated (Dodiya *et al.*, 2011). However, lipid peroxidation (LPO) and free radical generation in the renal

tubular cells have been suggested to be responsible for cisplatin-induced renal failure (Shimeda *et al.*, 2005; Sadzuka *et al.*, 1992; Baliga *et al.*, 1998). The xenobiotic-induced alterations in kidney functions are characterized by signs of injury, such as changes in urine volume, creatinine clearance, in GSH status, increase of LPO (Atessahin *et al.*, 2003). Cisplatin induces free radical production causing oxidative renal damage, possibly due to depletion of nonenzymatic and enzymatic antioxidant systems. Also, several studies have demonstrated the protective effect of antioxidants in cisplatin-induced nephrotoxicity (Somani *et al.*, 2000; Antunes *et al.*, 2001; Jiang *et al.*, 2007). The administration of antioxidants such as Vitamin E, Vitamin C, selenium and carotenoids, before or after treatment with CP has been used to protect or ameliorate against nephrotoxicity in human and animals (Giri *et al.*,

1998; De Martinis and Bianchi, 2001; Silva *et al.*, 2001; Naziroglu *et al.*, 2004). The grape is becoming increasingly popular as a fruit and is a significant source of nutritional antioxidants, such as polyphenols, anthocyanins as well as biologically active dietary components (El-Ashmawy *et al.*, 2007). Grape seed extract is a natural extract from the seed of *Vitis vinifera*. It is a rich source of one of the most beneficial groups of plant flavonoids, proanthocyanidins oligomers. These flavonoids exerts many-health-promoting effects including the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals (Ozer *et al.*, 2011).

The aim of this study was to investigate possible protective effects of exogenous GSE supplementation on CP-induced oxidative organ injuries and its effects on the levels of antioxidant enzymes, kidney function tests and lipid peroxidation, as well as histological changes.

MATERIALS AND METHODS

A total of 18 healthy male New Zealand white rabbits, weighing 2.5-3.0 kg, were used throughout this study. The animals purchased from the Veterinary Control and Research Institute, Elazig, Turkey, were kept under standard laboratory conditions (12 h light/12 h dark and 24±3°C) and fed with standard commercial rabbit chow (pellet form, in the sack, Elazig Food Company). Food and water were provided *ad libitum*. The experimental protocol was approved by the Veterinary Control and Research Institute Ethics Committee.

The rabbits were randomly divided into 3 equal groups; the first group served as control and received a single intraperitoneal dose of 0.9% saline. Rabbits from the second group were intraperitoneally injected with a single 5 mg kg⁻¹ body weight cisplatin dose. The CP dosage was selected on the basis of its effectiveness in inducing nephrotoxicity (Ozer *et al.*, 2011). The third group of rabbits was treated with GSE (proanthocyanidins dissolved in water) by oral gavage at the dose of 250 mg kg⁻¹ body weight (Yildirim *et al.*, 2011) for 6 consecutive days before and 6 consecutive days after the CP injection.

After CP GSE treatments, blood samples were collected from all animals by puncture of the jugular vein into sterile microtubes. After clotting at room temperature for 1 h, samples were centrifuged at 3000 g for 10 min at room temperature and sera were carefully harvested and stored at -20°C until analysed. Then, all rabbits were

decapitated under slight ether anaesthesia and kidney samples were immediately collected for biochemical and histopathological examinations. The kidney tissues were homogenized in glass-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenates.

Malondialdehyde (MDA) levels: The concentrations of malondialdehyde, reflecting the lipid peroxidation intensity were directly measured in the homogenates using the thiobarbituric-acid reaction described by Placer *et al.* (1966) and values were expressed as nmol g⁻¹ tissue.

Reduced GSH levels: The glutathione contents in kidney were measured at 412 nm using the method of Sedlak and Lindsay (1968) and expressed as nmol g⁻¹ tissue for kidney tissue.

Catalase activity: The kidney catalase activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (1983) and was expressed as katal g⁻¹ protein.

Glutathione peroxidase activity: The glutathione peroxidase activity was determined according to the method of Lawrence and Burk (1976) and expressed as U g⁻¹ protein for kidney tissue.

Protein determination: The protein concentration was also measured in the supernatants by the method of Lowry *et al.* (1951).

Kidney function tests: Serum urea, creatinine, Blood Urea Nitrogen (BUN) levels were measured using auto analyzer (Olympus AU 600, Japan).

Histopathology: At the end of the experiment, necropsy of the rabbits was performed and kidney tissue samples were fixed in 10% buffered neutral formalin. Paraffin-embedded blocks were routinely processed and 5 µm thick sections were stained with haematoxylin-eosin and examined under an optical microscope. (Olympus BX51) and 10 microscopic fields were examined in 20X magnification. Every fields were evaluated as severe (+++), moderate (++) , mild (+) and none (-).

Statistical analysis: Data are presented as Mean±Standard error of means (SEM). One-way analysis of variance and post hoc Duncan's test were used to

determine the differences between groups in terms of all studied parameters using the SPSS/PC computer program (version 12.0; SPSS, Chicago, IL, USA). The Kruskal Wallis test was used for the comparison between the control and experimental groups for histopathological examination. Differences were considered as significant when p-value was less than 0.05.

RESULTS

As reported in Table 1, the serum urea, BUN, creatinine were dramatically increased in rabbits from the CP group compared to the not treated controls ($p < 0.001$). by contrast, when animals received the oral GSE treatment, the enzyme activities remained similar to the control values.

The antioxidant/oxidant balance in kidney from rabbits treated with CP alone or coupled to GSE was summarized in Table 2.

It was observed that CP increase MDA levels ($p < 0.001$), the most important indicator of oxidative damage and decreased the activity of CAT ($p < 0.05$). Administration of CP did not caused statically a change in GSH levels and GSH-P_x activity ($p > 0.05$). Furthermore, it was found that the mean MDA content in kidney from GSE treated rabbits (CP/GSE group) was dramatically lowered compared to the animals treated with CP alone although it was remained significantly higher than in the healthy controls ($p < 0.001$).

The histopathological changes observed in the kidney are summarized in Table 3. Whereas no lesion was found in the healthy controls (Fig. 1c). The most significant changes in CP group are severe acute tubular

necrosis in proximal tubular epithelial cells, desquamation, cloudy swelling, tubular dilation, tubular epithelium karyomegali with formation of hyaline cylinders in tubular lumina (Fig. 1a). In addition to this view, mononuclear cell infiltration of the intertubular spaces, thickening of the basal membrane of glomerulus and postnecrotic fibrosis are the other changes. In the CP+GSE treatment group

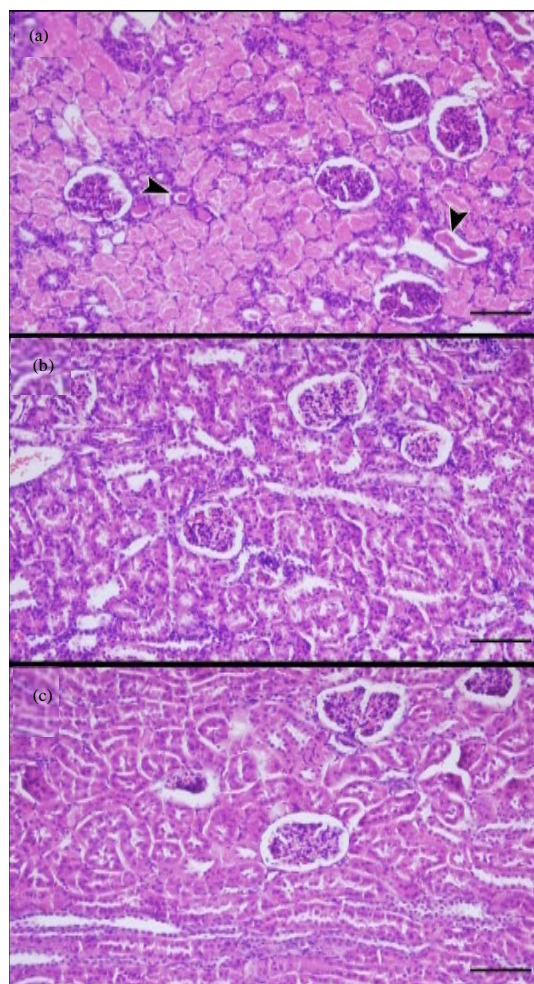


Fig. 1(a-c): Kidney histopathology in rabbits treated with cisplatin (5 mg kg⁻¹, intraperitoneally) and with cisplatin and grape seed extract (per os, 250 mg kg⁻¹ for 6 days before and 6 days after cisplatin injection). Picture a- Severe acute tubular necrosis with hyaline cylindrical formations (arrow heads). Cisplatin group. X 20 Picture b- Mild interstitial nephritis. Cisplatin+GSE group X 20 Picture c- Normal histological appearance of kidney (control group)

Table 1: Effects of cisplatin (5 mg kg⁻¹, intraperitoneally) and grape seed extract (GSE, per os, 250 mg kg⁻¹ for 6 days before and 6 days after)

Groups	Urea (mg dL ⁻¹)	Bun (mg dL ⁻¹)	Creatinine (mg dL ⁻¹)
Control	21.80±0.37 ^a	13.40±0.67 ^a	0.64±0.03 ^a
CP	52.80±3.62 ^b	27.20±2.15 ^b	1.60±0.17 ^b
CP+GSE	27.40±1.69 ^a	18.80±1.31 ^b	0.91±0.07 ^a
Groups	$p < 0.001$	$p < 0.001$	$p < 0.001$

Values with different letters with in column are significant different at $p < 0.01$

Table 2: Effects of cisplatin (5 mg kg⁻¹, intraperitoneally) and grape seed extract (GSE, per os, 250 mg kg⁻¹ for 6 days before and 6 days after cisplatin injection) treatments on antioxidant/oxidant equilibrium in kidney homogenates in rabbits

Groups	MDA umol g ⁻¹ tissue	CAT k g ⁻¹ protein	GSH umol g ⁻¹ tissue	GSH-P _x IU g ⁻¹ protein
	Control	7.03±1.40 ^a	255.60±14.24 ^b	1.89±0.02
CP	12.14±0.76 ^b	174.60±19.42 ^a	1.64±0.13	27.40±2.78
CP+GSE	8.24±0.42 ^a	200.80±15.95 ^a	1.97±0.22	26.60±1.69

Results are expressed as Mean±standard error of mean (SEM). Different superscripts in the same column indicate significant difference ($p < 0.05$ or more) between groups

Table 3: Effects of cisplatin (5 mg kg⁻¹, intraperitoneally) and grape seed extract (GSE, per os, 250 mg kg⁻¹ for 6 days before and 6 days after cisplatin injection) treatments on kidney histology in rabbits

Lesions	Groups				p-values
	Cisplatin	Cisplatin+GSE	Control	SH	
Acute tubular necrosis	2.17±0.40 ^a	0.67±0.33 ^b	0.00±0.00 ^b	0.27	0.001
Tubular dilatation	0.67±0.33 ^a	0.00±0.00 ^b	0.00±0.00 ^b	0.13	0.05
Formation of hyaline cylinders	1.33±0.56 ^a	0.83±0.31 ^{ab}	0.00±0.00 ^b	0.24	NS
Tubular epithelium karyomegali	0.67±0.21 ^a	0.67±0.21 ^a	0.00±0.00 ^b	0.12	0.05
Interstitial nephritis	0.67±0.33	0.50±0.22	0.00±0.00	0.14	NS
Thickening of the basal membrane of glomerulus	1.00±0.37 ^a	1.00±0.00 ^a	0.00±0.00 ^b	0.16	0.001
Tubular degeneration	1.33±0.42 ^a	1.83±0.31 ^a	0.00±0.00 ^b	0.25	0.001
Postnecrotic fibrosis	0.83±0.31 ^a	0.00±0.00 ^b	0.00±0.00 ^b	0.14	0.001

Different superscripts in the same column indicate significant difference (p<0.05 or more) between groups. SH: Standard error

degenerative changes were observed instead of severe necrotic changes in the proximal tubules (Fig. 1b). Tubular dilation was not observed in this group but intraluminal hyaline cylinders was found milder compared to the CP group.

DISCUSSION

Cisplatin (CP) is a potent chemotherapeutic agent that has gained widespread use against various malignant tumors in different experimental animals and in a variety of human malignancies (Kociba *et al.*, 1970; Prasad and Giri, 1994). However, high-dose therapy with CP is limited by its cumulative nephrotoxicity and neurotoxicity (Dwyer *et al.*, 1999). CP is toxic to the renal proximal tubules (Yao *et al.*, 2007). Recently, it has been postulated that oxidative stress and reactive oxygen species (ROS) are involved in the pathogenesis of CP-induced nephrotoxicity. It has been shown that superoxide anion (O₂⁻), H₂O₂ and hydroxyl radical (OH[•]) are involved in CP-induced nephrotoxicity (Yao *et al.*, 2007).

The ROS generated during normal cellular processes are immediately detoxified by endogenous antioxidants like GSH, CAT and so on but excessive ROS accumulation by CP causes an antioxidant status imbalance, leads to LPO and GSH depletion (Kim *et al.*, 2006). Oxidative stress resulting from cisplatin is defined by increased production of oxidative stress markers and decreased concentrations of antioxidants (Davis *et al.*, 2001; Ajith *et al.*, 2007). As a marker of oxidative stress, we evaluated CAT and GSH-P_x activity with GSH and MDA levels in kidneys. Our results in rabbit renal tissue demonstrated GSH depletion, enhanced GSH-P_x and increased LPO after CP injection, which is in accordance with earlier findings (Sugihara *et al.*, 1987). GSH protects the cell against the toxic effects of hydroxyl radicals and singlet oxygen. The decrease in GSH levels is associated with lower activity of glutathione related enzymes. Hence, more tissue damage due to the poor control on free radicals (Kandeel *et al.*,

2011). The decrease in the enzymatic antioxidant, CAT, activities in the kidney tissue may be responsible for increased levels of MDA (Ige *et al.*, 2011). The histological evaluation of the kidney preparations in pretreatment group also revealed a decrease CP-induced tubular necrosis. CP-induced renal damage is associated with increased renal vascular resistance and histologic damage to proximal tubular cells. In concordance with the results of earlier studies, our study on histological examination revealed major disruption in architecture of straight portion of proximal tubules due to CP toxicity. The toxicity was characterized by widespread degeneration of tubular architecture, tubular congestion, swelling and necrosis, luminal congestion and an infiltration of polymorphonuclear neutrophils. CP intoxication showed severe atrophy of glomerulus, which was apparent due to the reduction in its size. Marked dilation of proximal convoluted tubules with slogging of almost entire epithelium due to desquamation of tubular epithelium was evident. Cellular debris in the tubular lumen and increased tissue in the interstitium is also an indication of CP-induced renal necrosis. The changes obtained in the present study run parallel with the earlier report documented (Ravindra *et al.*, 2010). Histopathological inspection robustly supported the results of biochemical assays, henceforth confirming the GSE as ROS scavenger.

A second important parameter to take into account in the evaluation of the kidney damage produced by CP treatments is related to serum creatinine, urea and BUN levels. Creatinine is a metabolite of protein and is excreted in the urine via glomerular filtration and elevation of its levels in the blood is thus an indication of impaired kidney function (Bashandy and Al-Wasel, 2011). The CP injections reduced dramatically the renal function and produced an important increase in the level of creatinine, urea and BUN levels. However, the pretreatment with GSE reduces the observed damage in both cases and to an almost normal (control) level for urea, BUN and creatinine.

Free radical scavengers and antioxidants can ameliorate cisplatin-induced nephrotoxicity (Koyner *et al.*, 2008). During the recent past, a lot of focus has been given to dietary components. Majority of them being without side effects, thus taking the leading role in the fight against oxidative stress caused by different xenobiotics in humans. One such class of dietary components are the polyphenols found in various plant-derived foods. Polyphenols have been recently recognized as functionally active molecules, possessing antioxidant, anticancer, antimutagenic properties, as well as exerting protective effects against several other diseases (Nakamura *et al.*, 2001). Polyphenolic compounds are present in grape, which are powerful antioxidant properties, i.e. free radical scavenging activity (Monagas *et al.*, 2006).

In the present study, GSE treatment efficiently attenuated acute nephrotoxicity induced by a single injection of CP in rabbits. This was evidenced by significant improvement in the disturbed biochemical parameters (elevated BUN and serum creatinine levels and MDA level, with reduced kidney GSH level, increased CP and GSH-Px activities) and reduction of necrotic damage assessed by renal histopathological examination and scoring. The beneficial effects of GSE are well documented in earlier studies. Safa *et al.* (2010) demonstrated that pretreatment with red GSE protects against gentamicin-induced acute kidney injury as evident on tissue histology. Chis *et al.* (2009) found that long-term daily administration of GSE offers enhanced antioxidant potential and protection against tissue LPO and protein oxidation. Yildirim *et al.* (2011) indicate that the antioxidant GSE might have a protective effect against cisplatin-induced testicular damage and oxidative stress in rabbit. The mechanisms by which GSE ameliorate toxicity remains to be elucidated. We supposed they may inhibit LPO by scavenging free radicals and increasing intracellular concentration of glutathione.

In conclusion, this study is the first to report the effects of supplemental GSE in the rabbit kidney after cisplatin exposure. The present finding suggest that GSE protects against cisplatin nephrotoxicity and may be considered as a potentially useful candidate in the combination chemotherapy with cisplatin.

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